GCTTCCCGAGGCTCCGCACCAGCCGCGCTTCTGTCCGCCTGCAGGGCATTCCA
GAAAGATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTG
AACGCATTTACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTA
GCAATATGACAATTGAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGC
TGCACTAATTGTCTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGC
ATGGAGAGGAAGACCTGAAGGTTCAGCATAGTAGCTACAGACAGAGGGCCC
GGCTGTTGAAGGACCAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGA
TGTGAAATTGCAGGATGCAGGGGTGTACCGCTGCATGATCAGCTATGGTGGT
GCCGACTACAAGCGAATTACTGTGAAAGTCAATGCCCCATACAACAAAATCA
ACCAAAGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAACTGACATGT
CAGGCTGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGACCATC
AAGTCCTGAGTGGTAAGACCACCACCACCAATTCCAAGAGAGAGGAGAAGC
TTTTCAATGTGACCAGCACACTGAGAATCAACACAACAACTAATGAGATTTT
CTACTGCACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTG
GTCATCCCAGGTAATATTCTGAATGTGTCCATTAAAATATGTCTAACACTGTC
CCCTAGCACCTAGCATGATGTCTGCCTATCATAGTCATTCAGTGATTGTTGAA
TAAATGAATGAATGAATAACACTATGTTTACAAAATATATCCTAATTCCTCAC
CTCCATTCATCCAAACCATATTGTTACTTAATAAACATTCAGCAGATATTTAT
GGAATAAAAAAAAAAAAAAAAAAA

FIGURE 1

CGAGGCTCCGCACCAGCCGCGCTTCTGTCCGCCTGCAGGGCATTCCAGAAAGA
TGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTTGCTGAACGCATT
TACTGTCACGGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATATGAC
AATTGAATGCAAATTCCCAGTAGAAAAACAATTAGACCTGGCTGCACTAATTGT
CTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGAGAGGAAG
ACCTGAAGGTTCAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTTGAAGGAC
CAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGATGTGAAATTGCAGGAT
GCAGGGGTGTACCGCTGCATGATCAGCTATGGTGGTGCCGACTACAAGCGAAT
TACTGTGAAAGTCAATGCCCCATACAACAAAATCAACCAAAGAATTTTGGTTGT
GGATCCAGTCACCTCTGAACATGAACTGACATGTCAGGCTGAGGGCTACCCCA
AGGCCGAAGTCATCTGGACAAGCAGTGACCATCAAGTCCTGAGTGGTAAGACC
ACCACCACCAATTCCAAGAGAGAGGAGAAGCTTTTCAATGTGACCAGCACACT
GAGAATCAACACAACAACTAATGAGATTTTCTACTGCACTTTTAGGAGATTAGA
TCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGAACTACCTCTGGCACA
TCCTCCAAATGAAAGGACTCACTTGGTAATTCTGGGAGCCATCTTATTATGCCTT
GGTGTAGCACTGACATTCATCTTCCGTTTAAGAAAAGGGAGAATGATGGATGT
GAAAAAATGTGGCATCCAAGATACAAACTCAAAGAAGCAAAGTGATACACATTT
GGAGGAGACGTAATCCAGCATTGGAACTTCTGATCTTCAAGCAGGGATTCTCA
ACCTGTGGTTTAGGGGTTCATCGGGGCTGAGCGTGACAAGAGGAAGGAATGG
GCCCGTGGGATGCAGGCAATGTGGGACTTAAAAGGCCCAAGCACTGAAAATG
GAACCTGGCGAAAGCAGAGGAGGAGAATGAAGAAAGATGGAGTCAAACAGGG
AGCCTGGAGGGAGACCTTGATACTTTCAAATGCCTGAGGGGCTCATCGACGCC
TGTGACAGGGAGAAAGGATACTTCTGAACAAGGAGCCTCCAAGCAAATCATCC
ATTGCTCATCCTAGGAAGACGGGTTGAGAATCCCTAATTTGAGGGTCAGTTCCT
GCAGAAGTGCCCTTTGCCTCCACTCAATGCCTCAATTTGTTTTCTGCATGACTGA
GAGTCTCAGTGTTGGAACGGGACAGTATTTATGTATGAGTTTTTCCTATTTATTT
TGAGTCTGTGAGGTCTTCTTGTCATGTGAGTGTGGTTGTGAATGATTTCTTTTGA
AGATATATTGTAGTAGATGTTACAATTTTGTCGCCAAACTAAACTTGCTGCTTAA
TGATTTGCTCACATCTAGTAAAACATGGAGTATTTGTAAAAAAAAAAAAAAA

FIGURE 2

292 secreted (245 amino acids)

Signal/IgV/IgC/hydrophilic tail
(a) (b) (c) (d)

Ig cysteines in large bold

MRIFAVFIFMTYWHLLNA (signal)

FTVTVPKDLYVVEYGSNMTIE**C**KFPVEKQLDLAALIVYWEMEDKN
IIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQD
AGVYR**C**MISYGGADYKRITVKVNAPY (IgV)

NKINQRILVVDPVTSEHELT**C**QAEGYPKAEVIWTSSDHQVLSGKT
TTTNSKREEKLFNVTSTLRINTTTNEIFY**C**TFRRLDPEENHTAEL
VIP (IgC)

GNILNVSIKICLTLSPST (hydrophilic tail)

FIGURE 3

292 membrane (290 amino acids)

Signal/IgV/IgC/transmembrane (underlined)
plus cytoplasmic

Ig cysteines in large bold

MRIFAVFIFMTYWHLLNA (signal)

FTVTVPKDLYVVEYGSNMTIE**C**KFPVEKQLDLAALIVYWEMEDKN
IIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQD
AGVYR**C**MISYGGADYKRITVKVNAPY (IgV)

NKINQRILVVDPVTSEHELT**C**QAEGYPKAEVIWTSSDHQVLSGKT
TTTNSKREEKLFNVTSTLRINTTTNEIFY**C**TFRRLDPEENHTAEL
VIP (IgC)

ELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKC
GIQDTNSKKQSDTHLEET (transmembrane plus cytoplasmic)

FIGURE 4

AGATAGTTCCCAAAACATGAGGATATTTGCTGGCATTATATTCACAGCCTGC
TGTCACTTGCTACGGGCGTTTACTATCACGGCTCCAAAGGACTTGTACGTG
GTGGAGTATGGCAGCAACGTCACGATGGAGTGCAGATTCCCTGTAGAACG
GGAGCTGGACCTGCTTGCGTTAGTGGTGTACTGGGAAAAGGAAGATGAGC
AAGTGATTCAGTTTGTGGCAGGAGAGGAGGACCTTAAGCCTCAGCACAGCA
ACTTCAGGGGGAGAGCCTCGCTGCCAAAGGACCAGCTTTTGAAGGGAAAT
GCTGCCCTTCAGATCACAGACGTCAAGCTGCAGGACGCAGGCGTTTACTGC
TGCATAATCAGCTACGGTGGTGCGGACTACAAGCGAATCACGCTGAAAGTC
AATGCCCCATACCGCAAAATCAACCAGAGAATTTCCGTGGATCCAGCCACTT
CTGAGCATGAACTAATATGTCAGGCCGAGGGTTATCCAGAAGCTGAGGTAA
TCTGGACAAACAGTGACCACCAACCCGTGAGTGGAAGAGAAGTGTCACCA
CTTCCCGGACAGAGGGGATGCTTCTCAATGTGACCAGCAGTCTGAGGGTCA
ACGCCACAGCGAATGATGTTTTCTACTGTACGTTTTGGAGATCACAGCCAG
GGCAAAACCACACAGCGGAGCTGATCATCCCAGAACTGCCTGCAACACATC
CTCCACAGAACAGGACTCACTGGGTGCTTCTGGGATCCATCCTGTTGTTCC
TCATTGTAGTGTCCACGGTCCTCCTCTTCTTGAGAAAACAAGTGAGAATGCT
AGATGTGGAGAAATGTGGCGTTGAAGATACAAGCTCAAAAAACCGAAATGA
TACACAATTCGAGGAGACGTAAGCAGTGTTGAACCCTCTGATCGTCGATTG
GCAGCTTGTGGTCTGTGAAAGAAAGGGCCCATGGGACATGAGTCCAAAGAC
TCAAGATGGAACCTGAGGGAGAGAACCAAGAAAGTGTTGGGAGAGGAGCC
TGGAACAACGGACATTTTTTCCAGGGAGACACTGCTAAGCAAGTTGCCCAT
CAGTCGTCTTGGGAAATGGATTGAGGGTTCCTGGCTTAGCAGCTGGTCCTT
GCACAGTGACCTTTTCCTCTGCTCAGTGCCGGGATGAGAGATGGAGTCATG
AGTGTTGAAGAATAAGTGCCTTCTATTTATTTTGAGTCTGTGTGTTCTCACTT
TGGGCATGTAATTATGACTGGTGAATTCTGACGACATGATAGATCTTAAGAT
GTAGTCACCAAACTCAACTGCTGCTTAGCATCCTCCGTAACTACTGATACAA
GCAGGGAACACAGAGGTCACCTGCTTGGTTTGACAGGCTCTTGCTGTCTGA
CTCAAATAATCTTTATTTTTCAGTCCTCAAGGCTCTTCGATAGCAGTTGTTCT
GTATCAGCCTTATAGGTGTCAGGTATAGCACTCAACATCTCATCTCATTACA
ATAGCAACCCTCATCACCATAGCAACAGCTAACCTCTGTTATCCTCACTTCA
TAGCCAGGAAGCTGAGCGACTAAGTCACTTGCCCACAGAGTATCAGCTCTC
AGATTTCTGTTCTTCAGCCACTGTCCTTTCAGGATAGAATTTGTCGTTAAGAA
ATTAATTTAAAAACTGATTATTGAGTAGCATTGTATATCAATCACAACATGCC
TTGTGCACTGTGCTGGCCTCTGAGCATAAAGATGTACGCCGGAGTACCGGT
CGGACATGTTTATGTGTGTTAAATACTCAGAGAAATGTTCATTAACAAGGAG
CTTGCATTTTAGAGACACTGGAAAGTAACTCCAGTTCATTGTCTAGCATTAC
ATTTACCTCATTTGCTATCCTTGCCATACAGTCTCTTGTTCTCCATGAAGTGT
CATGAATCTTGTTGAATAGTTCTTTTATTTTTTAAATGTTTCTATTTAAATGATA
TTGACATCTGAGGCGATAGCTCAGTTGGTAAAACCCTTTCCTCACAAGTGTG
AAACCCTGAGTCTTATCCCTAGAACCCACATAAAAAACAGTTGCGTATGTTT
GTGCATGCTTTTGATCCCAGCACTAGGGAGGCAGAGGCAGGCAGATCCTG
AGCTCTCATTGACCACCCAGCCTAGCCTACATGGTTAGCTCCAGGCCTACA
GGAGCTGGCAGAGCCTGAAAAACGATGCCTAGACACACACACACACACACA
CACACACACACACACACACACACACCATGTACTCATAGACCTAAGTGCACC
CTCCTACACATGCACACACATACAATTCAAACACAAATCAACAGGGAATTGT

Figure 5

CTCAGAATGGTCCCCAAGACAAAGAAGAAGAAAAACACCAAACCAGCTCTA
TTCCCTCAGCCTATCCTCTCTACTCCTTCCTAGAAGCAACTACTATTGTTTTT
GTATATAAATTTACCCAACGACAGTTAATATGTAGAATATATATTAAAGTGTC
TGTCAATATATATTATCTCTTTCTTTCTTTCTTCCTTTCTTTCTTTCTTTCTTTC
TTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTCCTTCCTTCCTTCCTTCCTTC
CTTCCTTCCTTCCTTTCTTTCTTTCTTTCTTTTTTTCTGTCTATCTGTACCTAAA
TGGTTGCTCACTATGCATTTTCTGTGCTCTTCGCCCTTTTTATTTAATGTATG
GATATTTATGCTGCTTCCAGAATGGATCTAAAGCTCTTTGTTTCTAGGTTTTC
TCCCCCATCCTTCTAGGCATCTCTCACACTGTCTAGGCCAGACACCATGTCT
GCTGCCTGAATCTGTAGACACCATTTATAAAGCACGTACTCACCGAGTTTGT
ATTTGGCTTGTTCTGTGTCTGATTAAAGGGAGACCATGAGTCCCCAGGGTA
CACTGAGTTACCCCAGTACCAAGGGGGAGCCTTGTTTGTGTCTCCATGGCA
GAAGCAGGCCTGGAGCCATTTTGGTTTCTTCCTTGACTTCTCTCAAACACAG
ACGCCTCACTTGCTCATTACAGGTTCTCCTTTGGGAATGTCAGCATTGCTCC
TTGACTGCTGGCTGCCCTGGAAGGAGCCCATTAGCTCTGTGTGAGCCCTTG
ACAGCTACTGCCTCTCCTTACCACAGGGGCCTCTAAGATACTGTTACCTAGA
GGTCTTGAGGATCTGTGTTCTCTGGGGGGAGGAAAGGAGGAGGAACCCAG
AACTTTCTTACAGTTTTCCTTGTTCTGTCACATGTCAAGACTGAAGGAACAG
GCTGGGCTACGTAGTGAGATCCTGTCTCAAAGGAAAGACGAGCATAGCCGA
ACCCCCGGTGGAACCCCCTCTGTTACCTGTTCACACAAGCTTATTGATGAGT
CTCATGTTAATGTCTTGTTTGTATGAAGTTTAAGAAAATATCGGGTTGGGCAA
CACATTCTATTTATTCATTTTATTTGAAATCTTAATGCCATCTCATGGTGTTGG
ATTGGTGTGGCACTTTATTCTTTTGTGTTGTGTATAACCATAAATTTTATTTTG
CATCAGATTGTCAATGTATTGCATTAATTTAATAAATATTTTTATTTATTAAAAA
AAAAAAAAAAAAAAAA

Figure 5
(continued)

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKEDEQVIQFVAGEE
DLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGGADYKRITLKVNAPYRKINQRISV
DPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRSVTTSRTEGMLLNVTSSLRVNATANDVFYCTFWR
SQPGQNHTAELIIPELPATHPPQNRTHWVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRN
DTQFEET.

Figure 6

mB7-4 vs. hB7-4

69% identity

```
mB7-4   1  MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVVYWEKE  60
           MRIFA  IF    HLL AFT+T PKDLYVVEYGSN+T+EC+FPVE++LDL AL+VYWE E
hB7-4   1  MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEME  60

mB7-4  61  DEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDAGVYCCIISYGG 120
           D+ +IQFV GEEDLK QHS++R RA L KDQL  GNAALQITDVKLQDAGVY C+ISYGG
hB7-4  61  DKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG 120

mB7-4 121  ADYKRITLKVNAPYRKINQRI-SVDPATSEHELICQAEGYPEAEVIWTNSDHQPVSGKRS 179
           ADYKRIT+KVNAPY KINQRI  VDP TSEHEL CQAEGYP+AEVIWT+SDHQ +SGK +
hB7-4 121  ADYKRITVKVNAPYNKINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTT 180

mB7-4 180  VTTSRTEGMLLNVTSSLRVNATANDVFYCTFWRSQPGQNHTAELIIPELPATHPPQNRTH 239
            T S+ E  L NVTS+LR+N T N++FYCTF R  P +NHTAEL+IPELP  HPP  RTH
hB7-4 181  TTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTH 240

mB7-4 240  WVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGVEDTSSKNRNDTQFEET 290
            V+LG+ILL L V  T +  LRK  RM+DV+KCG++DT+SK ++DT  EET
hB7-4 241  LVILGAILLCLGVALTFIFRLRKG-RMMDVKKCGIQDTNSKKQSDTHLEET 290
```

Figure 7

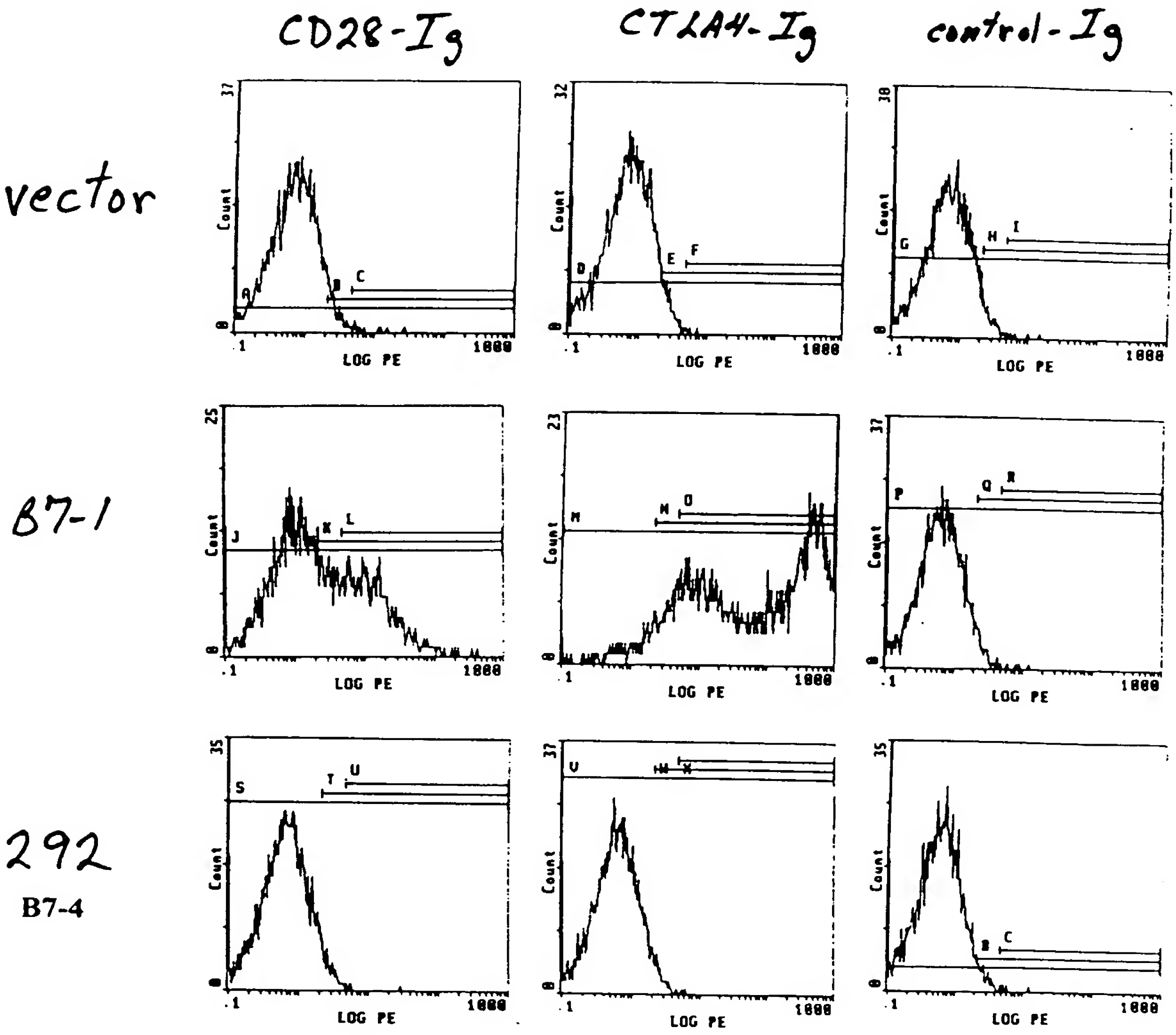
CD28-Ig
CTLA4-Ig
control-Ig
vector
B7-1
292
B7-4
Count
LOG PE
.1
1000
37
32
38
25
23
37
35
37
35
0
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
B
C

Figure 8

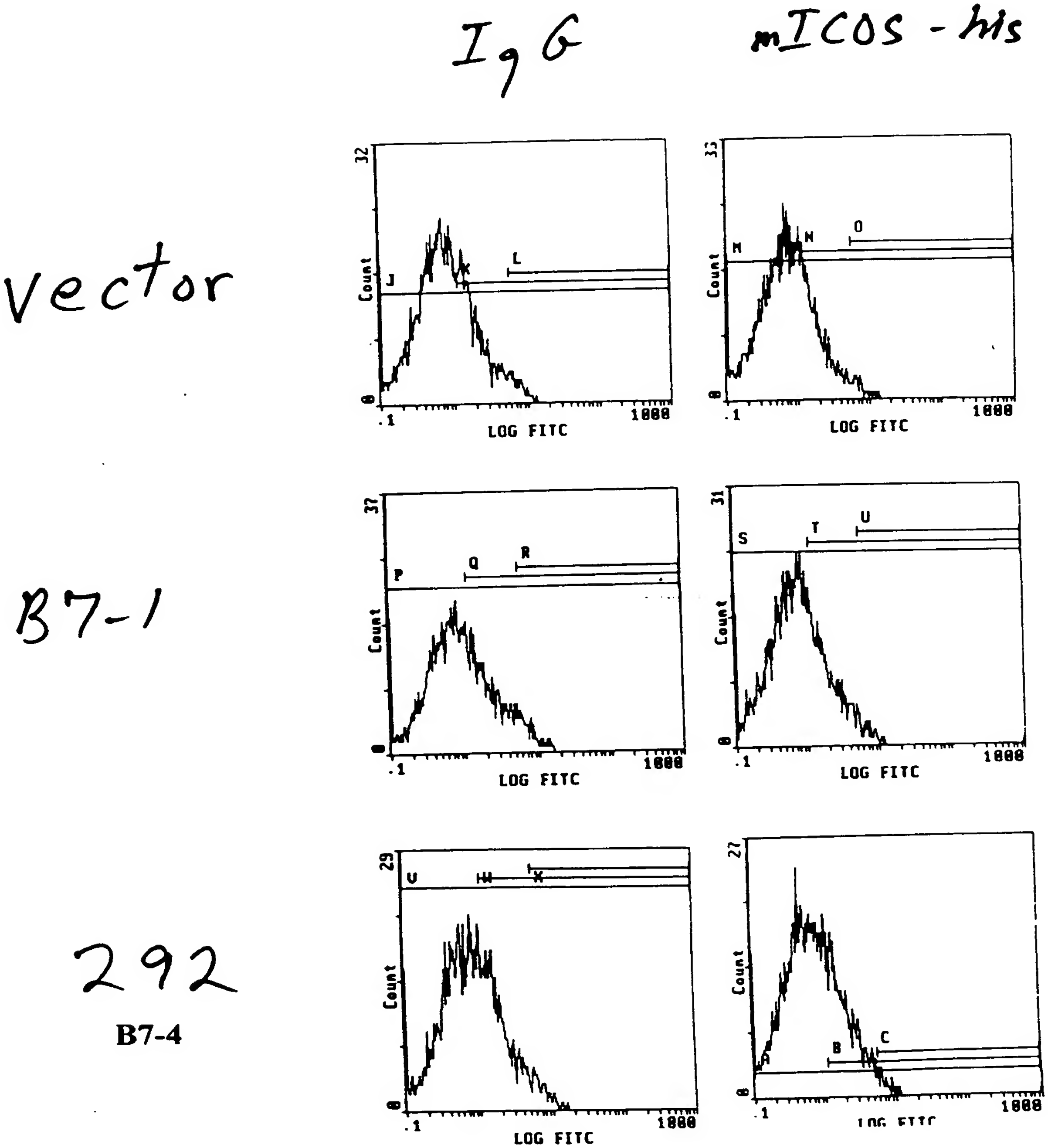
IgG
mICOS-his
vector
B7-1
292
B7-4
32
33
32
31
29
27
Count
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
A
B
C
0
.1
1000
LOG FITC

Figure 9

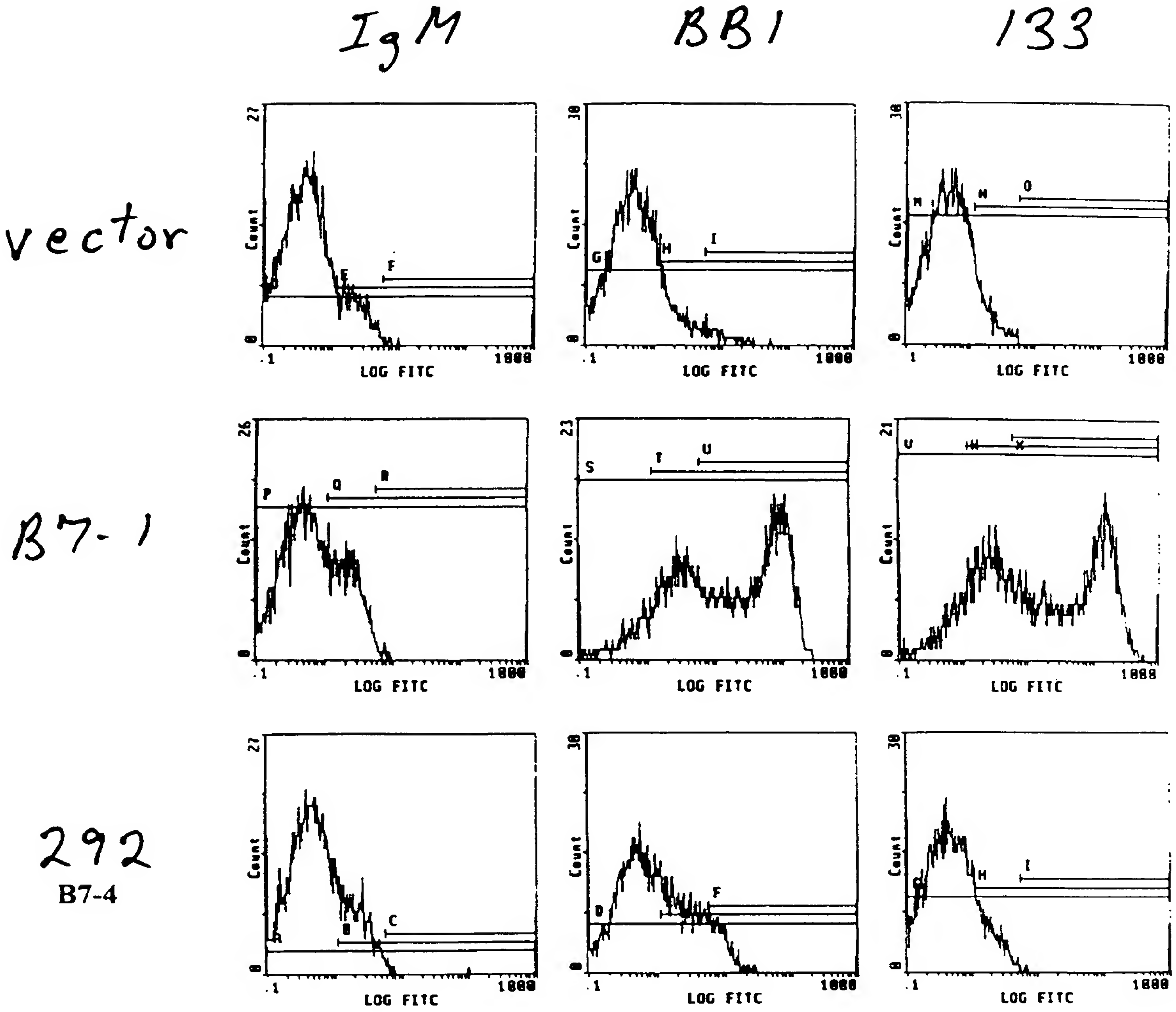
IgM
BB1
133
vector
B7-1
292
B7-4
Count
LOG FITC
.1
1
1000
27
30
26
23
21
0
E
F
G
H
I
M
N
O
P
Q
R
S
T
U
V
W
X
A
B
C
D

Figure 10

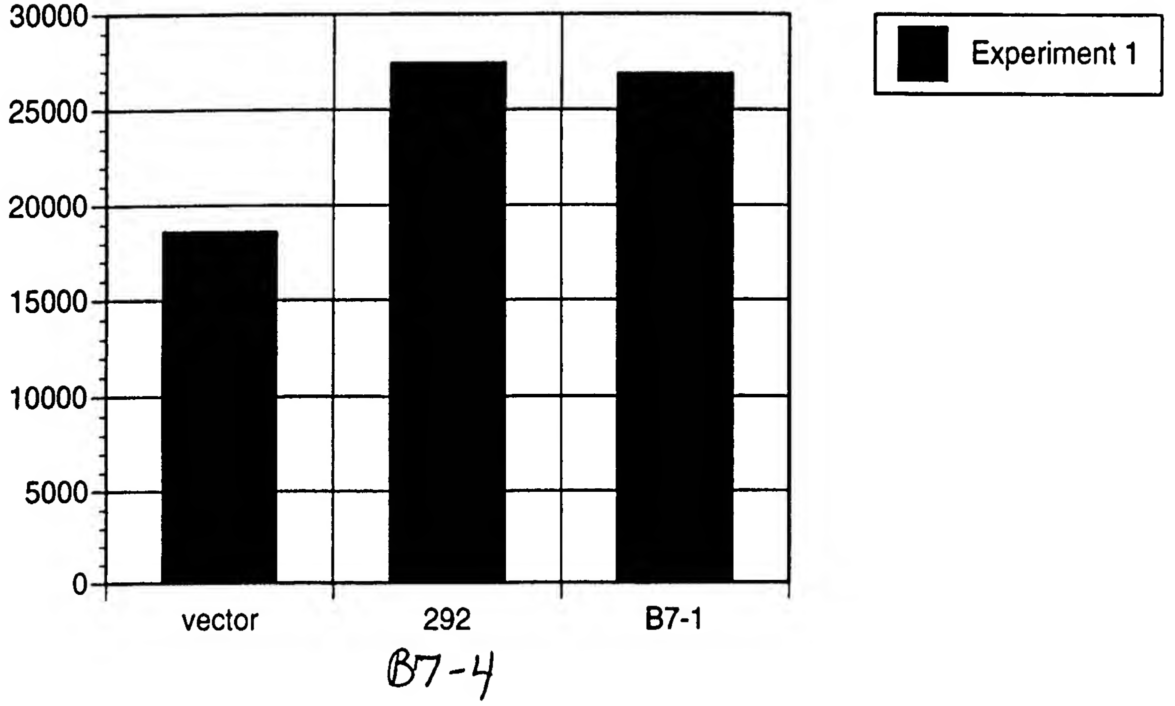
30000
25000
20000
15000
10000
5000
0
vector
292
B7-1
B7-4
Experiment 1

FIGURE 11

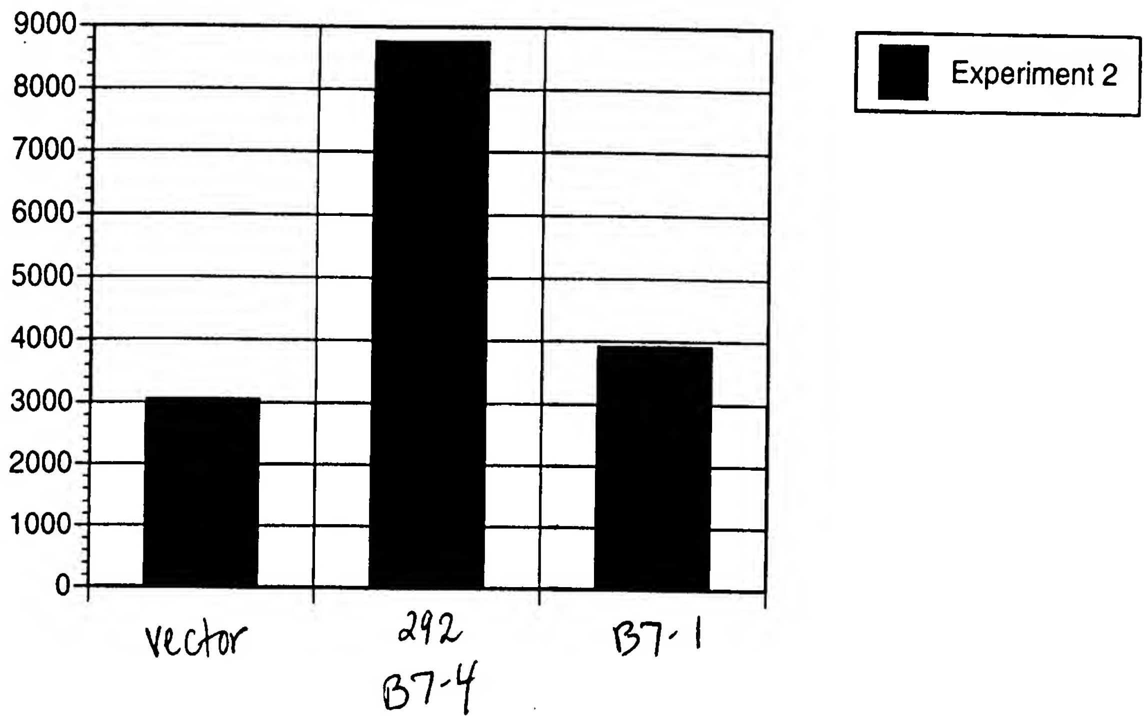
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
vector
292
B7-4
B7-1
Experiment 2

FIGURE 12